The effects of propionate and oxygen on the intracellular growth of the foodborne pathogen *Listeria monocytogenes*

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Background

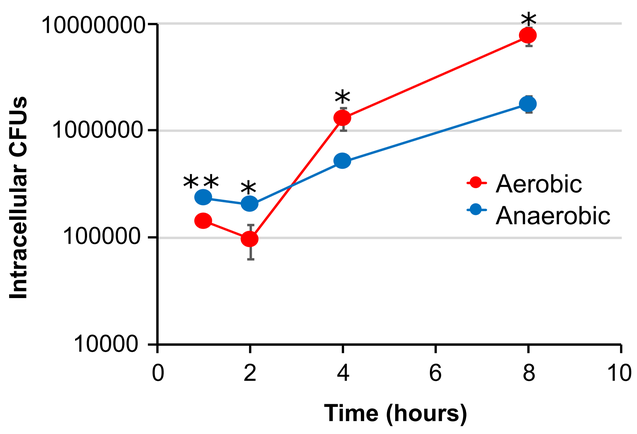
1. *Listeria monocytogenes* is a foodborne pathogen that causes illnesses in elderly and immunocompromised individuals by colonizing the human intestine. The fatality rate of *Listeria* infection is up to 30% in susceptible populations. Our ultimate research goal is to find a way to protect high-risk individuals against *Listeria* infection.

2. The human intestine is low in oxygen but rich in fermentation acids. Better understanding of how these unique conditions affect *Listeria* will enable us to explore the potential of chemical alterations in the gut as strategies to protect high-risk individuals.

3. Propionate is one of the major fermentation acids produced by the gut microbiota. In this study, we investigated the effects of changing oxygen conditions and propionate supplementation on growth and survival of *Listeria*.

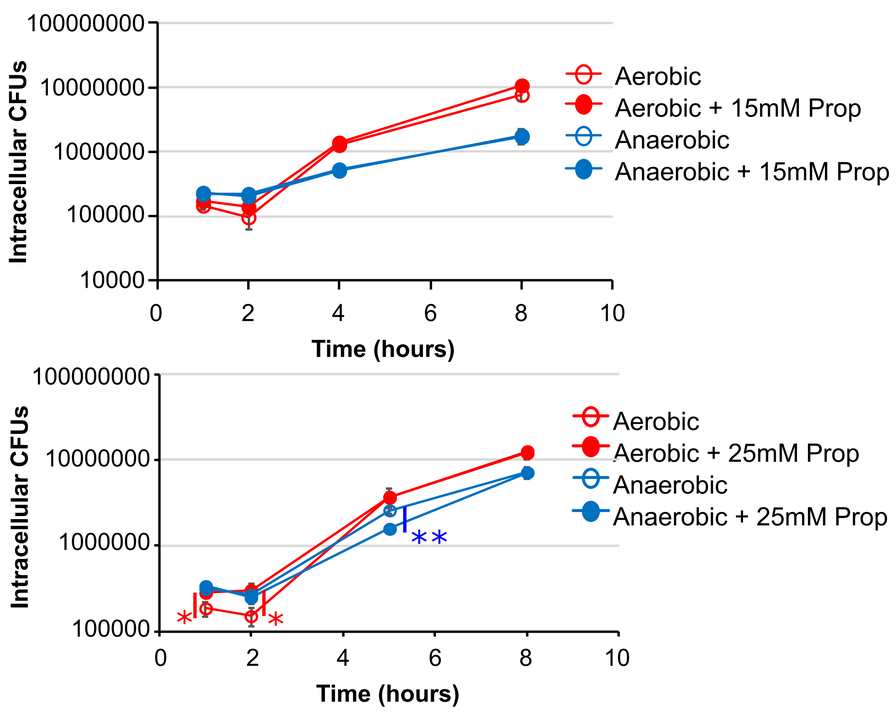
1. Anaerobically grown *Listeria* exhibited enhanced invasion but compromised intracellular growth compared to aerobically grown *Listeria*

We first tested intracellular growth of *Listeria monocytogenes* after overnight growth in BHI in aerobic or anaerobic conditions. We infected RAW 264.7 macrophages for 30 minutes at an MOI of 10. The cells were then treated with gentamycin to remove extracellular bacteria and lysed at different time points to enumerate intracellular bacteria.
Significance evaulated with a t-test: *=0.01-0.05, **=0.001-0.001, ***=0-0.001, NS=not significant.



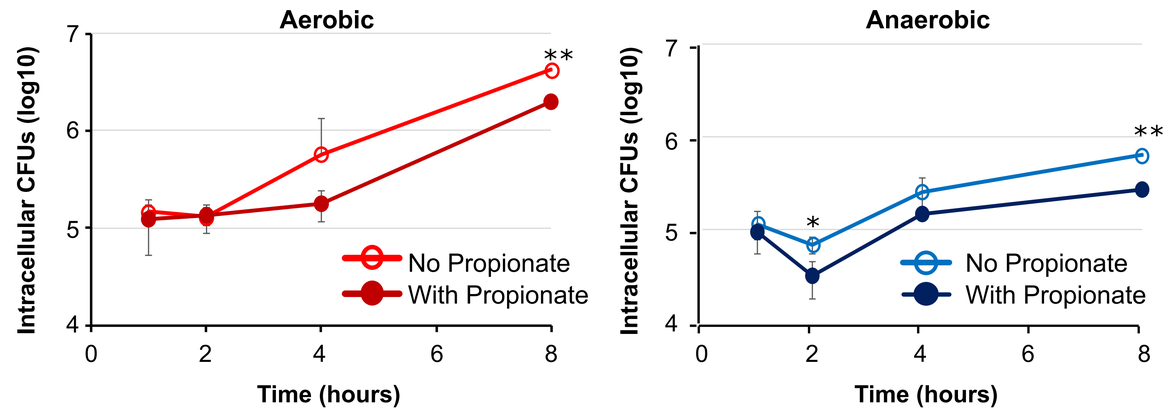
2. Bacterial exposure to propionate prior to infection did not impact intracellular growth

We next tested the effect of propionate on *Listeria* infections by infecting RAW 264.7 macrophages with *Listeria* grown aerobically or anaerobically with 15mM or 25mM propionate. Macrophages were infected for 30 minutes at an MOI of 10 and were then treated with gentamycin to remove extracellular bacteria and lysed at different time points to enumerate intracellular bacteria.



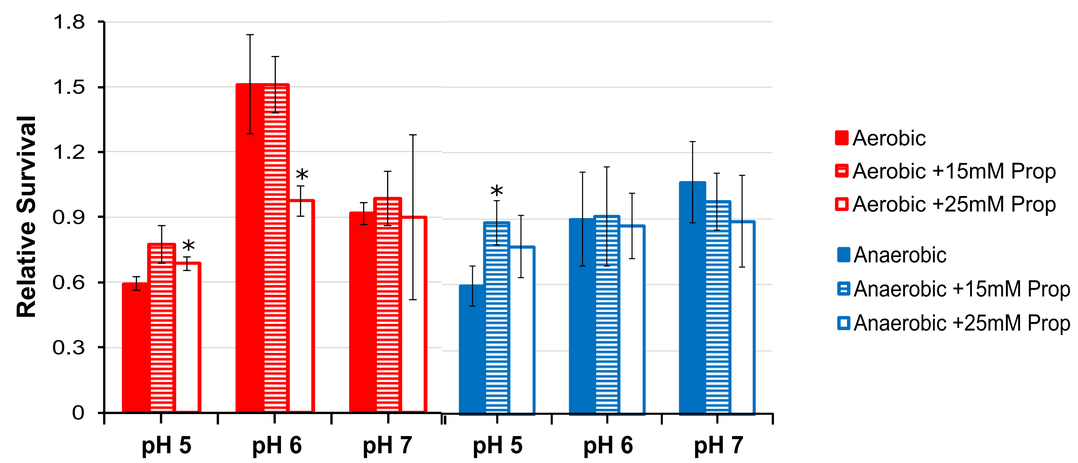
3. Presence of propionate during infection reduced *Listeria* intracellular growth

We next tested the effect of prolonged exposure to propionate both prior to and during *Listeria* infections. *Listeria* grown aerobically or anaerobically with or without 25mM propionate was used to infect RAW 264.7 macrophages for 30 minutes at MOI of 10. Propionate (5mM) was also added to the macrophages when they were seeded and consistently throughout infection.



4. Prior exposure to propionate altered *Listeria* survival in acidic conditions

Survival and escape from the acidifying phagosomes is critical during *Listeria* intracellular growth. To test the effects of propionate on *Listeria* survival in acidic conditions, we grew *Listeria* aerobically or anaerobically with or without 15mM or 25mM propionate and exposed them to phosphate-citric acid buffers at pH 4, 5, 6, or 7 for 1 hour. Colony forming units (CFUs) were counted before and after the 1 hour exposure to calculate percentage survival. No CFUs were recovered after 1 hour exposure at pH 4 (data not shown).



Conclusions and Future Direction

1. Growth in an anaerobic environment has a prolonged effect on *Listeria* pathogenesis.
2. Prior exposure to propionate, by itself, does not have a significant effect on *Listeria* intracellular growth. However, it does impact *Listeria* survival in acidic conditions.
3. When coupled with consistent propionate supplementation through infection, prior exposure to propionate has an inhibitory effect on *Listeria* intracellular growth.
4. We plan to continue this work by using immunofluorescence microscopy to visualize intracellular *Listeria* to determine how anaerobicity and propionate compromise intracellular growth.

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